REMARKS

This Reply is responsive to the Office Action dated October 22, 2002. Entry of the amendments and remarks submitted herein and reconsideration of the claimed subject matter pursuant to 37 CFR §1.112 is respectfully requested.

The specification has been amended above to incorporate reference to SEQ ID Nos. as requested in the Office Action. In addition, claims 10 and 15 have been canceled, and claims 17 and 19 have been amended to remove their dependency on the canceled claims. Claims 9, 12 and 14 have been amended to substitute the phrase "consisting of" for the word "if" for clarity purposes. No prohibited new matter is added by way of any of these amendments.

Turning now to the Office Action, in view of the objection to the Drawings, new Drawings are attached hereto for the Draftsperson's consideration.

The specification was objected to because the application contains sequences that are not accompanied by sequence identifiers. Applicants have carefully reviewed the specification and amended the disclosure where necessary to incorporate SEQ ID Nos. Withdrawal of the objection to the specification is respectfully requested.

The specification was also objected to because it is allegedly unclear whether the BFP variants included in the table on page 23 contain the mutations from the basic plasmid, S65T, H231L and a valine between Met1 and Ser2. Applicants respectfully note that the construction of the disclosed BFP variants is clearly described on pages 17-22 of the specification. For instance, as disclosed at page 17, lines 18-25, pUCBFP(101) was constructed by introducing the mutations of T65S, Y66H, and Y145F into the plasmid pUCGFP(101). This reverted the S65T mutation from the basic plasmid back to the wild type sequence at this site. The BFP coding sequence was then cloned into another vector to prepare BFP(201) as listed in the table on page

23. Thus, according to the specification, the BFP variants in the table on page 23 contain the H231L mutation and a valine between Met1 and Ser2, but contain a serine at position 65 in contrast to the basic plasmid, which contains a threonine mutation at this position. Reconsideration and withdrawal of the objection to the specification is respectfully requested.

Claims 8, 10, 11, 13 and 15-20 were rejected under 35 U.S.C. §112, first paragraph as allegedly lacking description in the specification. According to the Office Action, the claims encompass a genus of fluorescent proteins described by insufficient limitations on structure or function, and the specification allegedly discloses no identifying characteristics that would allow one to recognize a structure as exhibiting any fluorescence. The Office Action further argues that the specification teaches the structure of only a single representative species of such DNAs, and it would be allegedly unpredictable whether a protein is a fluorescent protein. Applicants respectfully traverse the rejection.

In the previous Reply filed July 10, 2002, Applicants noted that claims 8, 10, 11, 13, 15 and 16 recite that the claimed DNA's encode proteins having serine-tyrosine-glycine or serine-histidine-glycine at positions 65-67. The grouping serine-tyrosine-glycine is described in the specification as forming an imidazolidine ring oxidatively which serves as a chromophore (see paragraph bridging pages 2-3). Therefore, this grouping of amino acids forms an identifiable structure disclosed in the application as contributing to fluorescence.

Similarly, the application discloses numerous mutant proteins containing a mutation in the serine-tyrosine-glycine grouping substituting histidine for the tyrosine at position 65 (see Tables 4). Moreover, the application discloses that mutant proteins having this substitution (Tyr66His) retain fluorescence, and in some cases, exhibit a much higher level of fluorescence.

Accordingly, the grouping serine-histidine-glycine at positions 65-67 also forms an identifiable structure disclosed in the application as contributing to fluorescence.

The Examiner responded to Applicants' arguments by noting that, while the discussed triad contributes to fluorescence, it is not fluorescence itself. Further, the triad was known in the art and does not distinguish the claimed genus from the prior art. Therefore, the Examiner believes that "the correlation between the structure and the fluorescence common to all members of the genus is lacking from the description."

Applicant respectfully submits that each genus covered by the present claims is distinguished from the art by the newly described mutations. These newly described mutations constitute a structure common to all nucleic acids covered by the claims, and may be correlated to increased fluorescence as described in the specification (see page 7, lines 4-16). Thus, in addition to the chromophore triad as discussed above, the application correlates several new mutations with fluorescence. Accordingly, in contrast to what is alleged in the Office Action, the specification does describe identifying characteristics that would allow one to recognize a structure as exhibiting fluorescence, and describes a correlation between structure and fluorescence that is common to all members of each claimed genus.

Furthermore, Applicants strongly disagree with the statement in the Office Action that the specification teaches the structure of only a single representative species of the claimed DNAs. For instance, as noted in the Reply filed July 10, 2002, claim 11 is directed to a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1, with at least the mutations of Tyr145Phe, Phe64Leu, and Leu236Arg, said fluorescent protein also having serine-histidine-glycine corresponding to position Nos. 65-67 of SEQ ID No: 1 obtained by mutation Tyr66His. Applicants disclose actual reduction to practice of at least two

mutants - BFP(202) and BFP(205) - having the recited mutations (see Table 4). Moreover, BFP(205) contains the two additional mutations of Val163Ala and Ser175Gly (see Table 4 on page 23). Applicants also show a functional correlation with mutants having at least the three mutations recited, in that the species disclosed exhibit an extremely higher value of fluorescence at 37°C in comparison with BFP(201), which contains only the two mutations Tyr66His and Tyr145Phe (see Table 5). Thus, the application reasonably conveys to one of skill in the art of GFP proteins that there are many BFP proteins in the claimed genus other than BFP(202), which contains the four mutations recited, that exhibit fluorescence.

The Examiner responded to Applicants' arguments in the Office Action dated October 22, 2003, by arguing that the disclosed mutants do not support sufficient written description of the genus because the mutations represent a "miniscule percent of the structure that by itself is insufficient to impart fluorescence" and that there is no description of the rest of the protein structure. Applicants respectfully disagree because both the claims and the description disclose the rest of the protein structure as comprising the sequence of SEQ ID No. 1. While the Examiner is correct to note that the rejected claims are not limited to sequences comprising the newly described mutations, Applicants are not aware of any requirement in the Office Guidelines or in the case law that limits an applicant to the specific sequences disclosed in the specification.

Again, as noted in the Reply filed July 10, 2002, according to the Written Description Guidelines recently published by the Office (Federal Register, Vol. 66, No. 4, January 5, 2001) (of record), the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or

disclosed correlation between function and structure, or by a combination of such identifying characteristics (see page 1106 of the Federal Register publication, copy enclosed, column 3). According to the Guidelines, "what constitutes a 'representative number' [of species] is an inverse function of the skill in the art [and] depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus." Furthermore, the Guidelines stress that "[d]escription of a representative number of species does not require that the description be of such specificity that it would provide individual support for each species that the genus embraces." In fact, as stated on page 1101 of the Guidelines, at the tope of col. 3, "there is no basis for a *per se* rule requiring disclosure of complete DNA sequences or limiting DNA claims to only the sequence[s] disclosed" (with emphasis).

Applicant respectfully submits that the present application discloses actual reduction to practice of a representative number of species so as to justify the claimed genus. Furthermore, the present application discloses functional characteristics (increased fluorescence) coupled with a known or disclosed correlation between function and structure relating to the claimed proteins (*i.e.*, a chromophore ring combined with novel mutations leading to increased fluorescence) that would clearly convey to one of skill in the art that applicant was in possession of the claimed genus at the time of filing. The skill in the art relating to green fluorescence protein (GFP) mutants and mutagenesis procedures in general is quite developed as evidenced by the issued patents of record relating to mutants of GFP. Given that what constitutes a "representative number" of species is an "inverse function of the skill and knowledge in the art" as provided by the Written Description Guidelines, those of skill in the art relating to GFP mutants would

recognize in applicant's disclosure a genus of DNA's encoding mutant GFP proteins having at least the mutations recited in the claims.

In any case, Applicants respectfully submit that this rejection is inconsistent with previous prosecution in that claims containing the language "at least the mutations of," and hence reading on a broader genus of proteins, have already issued in U.S. Patent No. 6,194,548. For instance, claim 1 of U.S. Patent is directed to "A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence comprising at least the mutations of Phe64Leu, Val163Ala, and Ser175Gly." Claim 8 of the present application is directed to DNAs encoding the same genus of proteins. The Examiner has provided no reasons as to why the specification supports a claim to the genus of proteins but does not describe a claim to DNAs encoding such proteins.

In view of the actual reduction to practice of several species within the claimed genus, the functional correlation between the structure of these mutants and a higher fluorescence, and the fact that the skill in the art of GFP mutations is well developed such that the skilled artisan would immediately envision a genus of mutants having the recited mutations, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, for alleged lack of written description.

Claims 8, 10, 11, 13 and 15-20 were also rejected under 35 U.S.C. §112, first paragraph, because the specification, while being allegedly enabling for a fluorescent protein having the amino acid of SEQ ID No. 1 consisting of the mutations recited in the claims, allegedly fails to enable SEQ ID No. 1 comprising other mutations in addition to these mutations. In particular, while the Examiner agrees that recombinant and mutagenesis techniques are known, she does not believe that it is "routine in the art to screen large numbers of mutated proteins where the

expectation of obtaining similar activity is unpredictable based on the instant disclosure." Applicants respectfully traverse the rejection.

Applicants noted in the previous Reply filed July 10, 2002, that the specification discloses more than one mutant falling within the scope of each claim. For instance, taking claim 11 as an example, in addition to mutant BFP(202), which contains the four mutations recited, Applicants also disclose BFP(205), into which the additional mutations of Val163Ala and Ser175Gly have been introduced (see Table 4 on page 23). Thus, the specification is enabling for other species for claim 11 than a mutant "consisting of" the four recited mutants, because the specification demonstrates actual reduction to practice of other proteins containing further mutations in addition to those recited.

Furthermore, the isolation of more than one protein within the claimed genus provides an expectation that one of skill in the art may successfully identify further mutant proteins within the genus using the methods disclosed in the specification. Indeed, the Application discloses at pages 18-20 a method for randomly introducing mutations into a GFP sequence using Mutagenic PCR, wherein random mutants are screened for increased fluorescence in *E. coli* following UV irradiation. The present inventors isolated and sequenced ten different mutant clones using this procedure, and identified several mutations as listed in Table 1 on page 28. One of skill in the art upon reading applicant's disclosure could readily perform random Mutagenic PCR on BFP(202), for instance, which contains the recited four mutations, and readily screen for random mutants that display fluorescence using the assay disclosed in the specification. Moreover, the skilled artisan would have an expectation of success that proteins containing additional mutations could be isolated given Applicants' identification of BFP(202) and BFP(205) as described above.

The Examiner responded to Applicants' arguments in the Office Action dated October 22, 2002, by arguing that Mutagenic PCR and other techniques are known in the art and are not novel to the instant invention. Applicant fails to see, however, what this argument has to do with the rejection at hand. The rejected claims are not directed to methods for identifying novel mutations, rather, the claims are directed to DNAs containing novel mutations. A novel product is patentable even though it may have been isolated using known methods.

The Examiner further argues that Applicants have already collected all the mutants with the requisite properties obtained by the described method, and that this does not allow one to predict what further mutations are permissible. Applicants strongly disagree with the Examiner's position. Applicants have identified several specific mutations in the sequence of GFP and BFP that lead to increased fluorescence and have claimed DNA encoding proteins comprising these specific mutations. Applicants reasonably expect that variants of these specific GFP and BFP mutants containing sequence changes at other positions could readily be obtained that still retain the functional properties associated with the specific mutations that are claimed.

It is important to note that a given mutant GFP or BFP protein containing the claimed mutations simply needs to retain the function of increased fluorescence that is already exhibited by a protein having the base sequence and specific mutation claimed. In a mutagenesis experiment using the claimed DNA sequences, this will constitute most of the mutants obtained in the screen. In fact, it would be highly irregular if one of ordinary skill in the art was not able to identify BFP and GFP variants containing further mutations, *i.e.*, conservative mutations, starting with the sequence of the mutants as claimed, that do not retain the function of increased fluorescence exhibited by the claimed mutants. The Examiner appears to be arguing that any

further mutation of the sequence of the claimed mutant will destroy the functionality of increased fluorescence, and this is simply not true.

The Examiner further asserts that claims comprising the specific mutations and further limited to sequences that are 95% identical to SEQ ID No. 1 would be enabled. Applicants fail to understand this logic, however, since a mutant having a single loss of function mutation would be 95% identical to SEQ ID No. 1 and not have the properties of the claimed mutant. The present claims are limited to "fluorescent" proteins having the claimed mutations, which, in contrast to the Examiner's suggested language, requires that all residues of SEQ ID No. 1 required for fluorescence be present.

Thus, in contrast to what was alleged in the Office Action dated October 22, 2002, there is a strong expectation that one may successfully obtain other mutants demonstrating fluorescence that contain further mutations in addition to those recited in the claims. Further, Applicants have disclosed methods whereby the skilled artisan could readily screen large numbers of random mutants in order to identify those exhibiting or retaining increased fluorescence. Given that the disclosure provides an expectation of success in isolating further mutants within the claimed genus and a screening method for identifying further mutants, the specification is enabling for the full scope of the claims.

In any case, Applicants respectfully submit that this rejection is inconsistent with previous prosecution in that claims containing the language "at least the mutations of," and hence reading on a broader genus of proteins, have already issued in U.S. Patent No. 6,194,548. For instance, as discussed above in response to the written description rejection, claim 1 of U.S. Patent is directed to "A fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1 in the Sequence Listing, said sequence comprising at least the mutations of Phe64Leu,

Val163Ala, and Ser175Gly." Claim 8 of the present application is directed to DNAs encoding the same genus of proteins. The Examiner has provided no reasons as to why a claim to the genus of proteins is enabled by the specification but a claim to DNAs encoding such proteins is not.

In view of all the remarks submitted above, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, for lack of enablement is respectfully requested.

Claims 9, 12 and 14 with dependent claims 17-20 were rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness. According to the Office Action, it is unclear whether the word "with" in these claims implies open or closed language. Without necessarily agreeing with the rejection, Applicants have amended claims 9, 12 and 14 above to include the more familiar terminology "consisting of." Applicants note that the claims have already been examined on the assumption that the claims contain closed language.

Claim 15 was rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Siemering *et al.* Without necessarily agreeing with the rejection and solely for the purpose of expediting an allowance of the present application, claim 15 has been canceled. Withdrawal of the rejection is respectfully requested.

Claim 10 was rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Tsien *et al.* Without necessarily agreeing with the rejection and solely for the purpose of expediting an allowance of the present application, claim 10 has been canceled. Withdrawal of the rejection is respectfully requested.

Claims 8 and 9, with dependent claims 17 and 18, were rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Siemering *et al.* in view of Thastrop *et al.* According to the Office Action, it would have been obvious to combine the mutations Val163Ala/Ser175Gly as

taught by Siemering *et al.* with the Phe64Leu mutation as taught by Thastrop *et al.* with a reasonable expectation of success of at least a cumulative effect "because both mutants retain their respective properties when combined with other mutations. Applicants respectfully traverse the rejection.

According to MPEP 2143.01, the test for obviousness is what the combined teachings of the prior art would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts. Where the teachings of two or more prior art references conflict, the Examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which one reference might accurately discredit another. *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991).

Applicants respectfully submit that one of ordinary skill in the art would not be motivated to use the Phe64Leu mutation of Thrastup *et al.* to achieve increased fluorescence in view of other teachings in the prior art, for instance, the Ellenberg *et al.* and Angres & Green references submitted in the Information Disclosure Statement dated July 10, 2002. Angres & Green (published by Clontech Laboratories) teaches that excitation of an enhanced BFP (EBFP) variant comprising the mutation Phe64Leu "turned out to be quite destructive to the chromophore and photobleaching was a considerable problem" (see first full paragraph). This observation was confirmed by the authors of the Ellenberg *et al.* reference, who state that, while the commercially engineered variant EBFP shows good spectral separation from green emitting variants, it is "dim" and "photobleaches extremely rapidly" (see paragraph bridging pages 842-44).

Thus, in contrast to the argument set forth in the Office Action, one of ordinary skill in the art would not have been motivated to use the Phe64Leu mutation disclosed by Thrastup *et al.*

to achieve improved fluorescence since the EBFP mutant designed by Clontech containing this mutation was reportedly "dim" and exhibited considerable photobleaching. Further, there would have been no reason to believe that the Phe64Leu mutation would lead to improved fluorescence when combined with other mutations, since it did not lead to improved fluorescence when combined with the Ser65Thr, Tyr66His and Tyr145Phe mutations in the EBFP mutant. Reconsideration and withdrawal of the §103 rejection based on Siemering *et al.* and Thrastup *et al.* are respectfully requested.

Claim 10, with dependent claims 19 and 20, was rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Tsien *et al.* in view of Thrastup *et al.* According to the Office Action, it would have been obvious to combine the Phe64Leu mutation taught by Thrastup *et al.* with the Tyr66His and Tyr145Phe mutations taught by Tsien *et al.* to achieve the mutant recited in claim 10, since Thrastup teaches that addition of Phe64Leu to the original BFP resulted in improved fluorescence.

At the outset, Applicants respectfully note that claim 10 was rejected under 35 U.S.C. §102(e) based on Tsien *et al.* alone, so it is not clear why the Examiner relies on Thrastup *et al.* to provide the Phe64Leu mutation. In any case, claim 10 has been canceled. Therefore, the rejection appears to be moot. Withdrawal of the rejection is respectfully requested.

Claim 16, with dependent claims 19 and 20, was rejected under 35 U.S.C. §103(a) as being unpatentable over Tsien *et al.* and Siemering *et al.* According to the Office Action, it would have been obvious to combine the Tyr66His and Tyr145Phe mutations taught by Tsien *et al.* and the Val163Ala and Ser175Gly mutations taught by Siemering *et al.* to achieve the claimed mutant, since the mutations of Siemering *et al.* are disclosed as providing enhanced thermostability, and one would have a reasonable expectation of a cumulative effect since both

sets of mutations retain their respective properties when combined with other mutations. Applicants respectfully traverse the rejection.

As noted above with regard to the first §103 rejection, according to MPEP 2143.01, the test for obviousness is what the combined teachings of the prior art would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts. Where the teachings of two or more prior art references conflict, the Examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which one reference might accurately discredit another. *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991).

Applicants respectfully submit that one of ordinary skill in the art would not be motivated to use the Tyr66His and Tyr145Phe mutations of Tsien *et al.* to achieve improved fluorescence in view of other teachings in the prior art, for instance, the Ellenberg *et al.* and Angres & Green references submitted in the Information Disclosure Statement dated July 10, 2002. Angres & Green (published by Clontech Laboratories) teaches that excitation of an enhanced BFP (EBFP) variant comprising the mutations Tyr66His and Tyr145Phe "turned out to be quite destructive to the chromophore and photobleaching was a considerable problem" (see first full paragraph). This observation was confirmed by the authors of the Ellenberg *et al.* reference, who state that, while the commercially engineered variant EBFP shows good spectral separation from green emitting variants, it is "dim" and "photobleaches extremely rapidly" (see paragraph bridging pages 842-44).

Thus, in contrast to the argument set forth in the Office Action, one of ordinary skill in the art would not have been motivated to use the Tyr66His and Tyr145Phe mutations disclosed by Tsien *et al.* to achieve improved fluorescence since the EBFP mutant designed by Clontech

containing these mutations was reportedly "dim" and exhibited considerable photobleaching. Further, there would have been no reason to believe that the Tyr66His and Tyr145Phe mutations would lead to improved fluorescence when combined with other mutations, since they did not lead to improved fluorescence when combined with the Phe64Leu and Ser65Thr mutations in the EBFP mutant. Reconsideration and withdrawal of the §103 rejection based on Tsien *et al.* and Siemering *et al.* are respectfully requested.

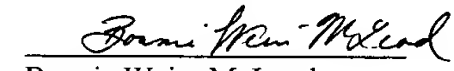
This reply is fully responsive to the Office Action dated October 22, 2002. Therefore, a Notice of Allowance is next in order and is respectfully requested.

Except for issue fees payable under 37 CFR §1.18, the commissioner is hereby authorized by this paper to charge any additional fees during the pendency of this application including fees due under 37 CFR §1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 CFR §1.136(a)(3).

If the Examiner has any further questions relating to this Reply or to the application in general, she is respectfully requested to contact the undersigned by telephone so that allowance of the present application may be expedited.

Respectfully submitted,
Morgan, Lewis & Bockius LLP

Dated: April 21, 2003

By: 
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APPENDIX

The following amendments were presented above:

IN THE SPECIFICATION:

The paragraph beginning at page 3, line 20, was replaced with the following amended paragraph:

--Recently, a mutant of GFP into which the mutations of Y66H and Y145F were introduced and which had different wavelength characteristics (it is also referred to as "Mutant," and its amino acid sequence (SEQ ID No. 15) is described below [with the above-mentioned mutations shown as underlined]) was developed. This is referred to as "BFP (Blue Fluorescent Protein)," because it emits blue fluorescence by UV excitation. (R. Heim et al. Curr. Biol. 6: 178-182 (1996); R. Heim et al. Proc. Natl. Acad. Sci. USA 91: 12501-04 (1994).) In the present specification, the term "BFP protein" refers to a protein that emits blue fluorescence when excited by ultraviolet-blue light and that, then, does not require an energy source such as a special substrate or ATP. However, such BFP had a problem in that it experienced severe fading as compared to GFP and was difficult to be observed under the microscope or the like. As used herein to designate mutation, the position of the mutation is expressed by a specific amino acid number in the sequence of the above-mentioned wild type; the amino acid prior to its mutation is described preceding the number and the mutated amino acid is to be described following the number.--

The paragraph beginning at page 14, line 18, was replaced with the following amended paragraph:

--In this invention, a DNA portion encoding GFP of pGFP-Cl (available from Clontech Inc.) was replaced by a DNA of GFP derived from phGFP-S65T (available from Clontech Inc.), which served as a basic plasmid (hereinafter referred to as "phGFP(101)-Cl"). The vector is meant for expression in mammalian cells and its full base sequence including the vector part is known in the art. The corresponding amino acid sequence (SEQ ID No. 14) is set forth below.--

The paragraph beginning at page 20, line 13, was replaced with the following amended paragraph:

--The site-directed mutation introduction methods are not particularly limited, and for example, the protocol for a Quick Change Kit from Stratagene Inc. was followed. The oligonucleotides shown in Table 2 below (SEQ ID Nos. 2-13, respectively) were used as primers and the plasmid (about 0.03 μg) obtained by subcloning GFP or [BFPcDNA] BFP cDNA into the HindIII site of a pUC18 or pQE30 vector was used as a template. The concrete PCR conditions are preferably as follows: 16 cycles at 95 °C for 30 sec, 55 °C for 1 min, and 68 °C for 10 min.--

IN THE CLAIMS

Claims 10 and 15 were canceled.

The following claims were amended as shown:

9. (Twice Amended) DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1, said sequence consisting of [with] the three mutations Phe64Leu, Val163Ala and Ser175Gly.

12. (Twice Amended) DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1, said sequence consisting of [with] the four mutations Tyr66His, Tyr145Phe, Phe64Leu and Leu236Arg.

14. (Twice Amended) DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID No. 1, said sequence consisting of [with] the six mutations Tyr66His, Tyr145Phe, Phe64Leu, Val163Ala, Ser175Gly and Leu236Arg.

17. (Amended) A method of visually analyzing gene expression or protein localization in a cell comprising the steps of:

(a) providing a cell transected with a vector comprising the DNA of any of claims 8[,] or 9 [or 15];

(b) culturing the cells under conditions to express a fluorescent protein encoded by the vector; and

(c) detecting the presence of the fluorescent protein.

19. (Amended) A method of visually analyzing gene expression or protein localization in a cell comprising the steps of:

(a) providing a cell transected with a vector comprising the DNA of any of claims [10,] 11, 12, 13, 14 or 16;

(b) culturing the cells under conditions to express a fluorescent protein encoded by the vector; and

(c) detecting the presence of the fluorescent protein.